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Proton NMR relaxation study of swelling and gelatinisation process in rice starch–water samples

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Abstract

Proton transverse magnetization decay curves of rice flour starch–water samples were measured and analysed for the presence of four components in the relaxation curve. T_2 values were interpreted on the basis of the diffusive and chemical exchange model that provided evidence for extra granular bulk water and three more water populations whose relaxation rate is governed by diffusive and chemical exchange with starch components. The analysis of relaxation data provided information on dynamics of water molecules as well as on the size and dispersion of diffusive domains. Furthermore, by measuring solid to liquid ratio, transverse and longitudinal relaxation curves of starch–water mixtures at increasing temperatures – from 20 to 77 °C – swelling and gelatinisation processes were monitored.
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Keywords: Rice starch; Low-resolution NMR; Proton relaxation times; Swelling; Gelatinisation

1. Introduction

Besides providing a major source of energy in foods, starch is important because of the many starch-based ingredients developed, principally used to take up water, and to produce viscous fluids and/or gels to impart the desired textural quality to food products. In addition, water immobilization in foods prevents growth of micro-organisms, thereby contributing to a longer shelf life. In order to optimise processing operations and obtain the desired quality of starch-based foods, an understanding of gelatinisation process, i.e., the break up and partial dissolution of starch granule upon heating in the presence of water, is required. That is why it is crucial to investigate the distribution and dynamic state of water within starch granules, during thermal treatments and other physical or chemical processing.

So far, different techniques have been used to study the dynamic state of starch and water components in native starch products and during their processing. Differential scanning calorimetry (DSC) has been extensively used to study gelatinisation of potato starch granules (Blanshard, 1987; Donovan, 1979; Muhr, Blanshard, & Blates, 1984), glass and melting transition in starch suspensions and gels (Liu & Lelievre, 1991; Liu, Lelievre, & Ayoungchee, 1991), and in blends of wheat and rice starch (Liu & Lelievre, 1992). X-ray diffraction has been used to monitor crystallization of amylose–lipid complexes during maize starch melting (Le Bail et al., 1999) and to study starch gelatinisation (Liu et al., 1991). Small angle neutron scattering (SANS) has been used to probe water distribution inside waxy maize granules during gelatinisation (Jenkins & Donald, 1998; Tang, Brun, & Hills, 2001). Changes in apparent viscosity, determined by rheological methods, have been used (Kubota, Hosokawa, Suzuki, & Hosaka, 1979; Okechukwu, Rao, Ngoddy, & McWatters, 1991) to quantify gelatinisation kinetics in rice and cowpea starches and in cowpea flour. The kinetics of water dispersion during gelatinisation of dilute suspensions of maize starch has been moreover studied by analysing changes in electrical conductance data recorded continuously with time (Karapantsios, Sakonidou, & Raphaelides, 2002).

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Finally, nuclear magnetic resonance (NMR) has been employed to study the state of water in starch granules by using different techniques: continuous wave (CW) (Leckert, 1976; Mousseri, Steinberg, Nelson, & Wei, 1974); magical angle spinning (MAS) (Morgan, Furneaux, & Larsen, 1995; Tang & Hills, 2003; Tanner, Hills, & Parker, 1991); two-dimensional ^{1}H–^{13}C wide line separation solid-state (WISE) (Kulik, Chris de Costa, & Haverkamp, 1994); deuterium spectra (Chatakanonda, Dickinson, & Chinachioti, 2003; Li, Dickinson, & Chinachoti, 1998; Tang, Godward, & Hills, 2000); proton relaxation studies (Choi & Kerr, 2003a, 2003b; Hills & LeFloc'h, 1994; Kim, Yoo, Cornillon, & Lim, 2004; Lewen, Paeschke, Reid, Molitor, & Schmidt, 2003) and pulsed field gradient measurements (Gomi, Fukuoka, Minori, & Watanabe, 1998; Hills, Godward, Manning, Biechlin, & Wright, 1998; Lewen et al., 2003). NMR is undoubtedly the most suitable technique for studying the dynamic state of water in heterogeneous systems. In fact, the relationship between water proton relaxometry and distribution of water in randomly packed particulate beds has been explored in a variety of heterogeneous systems like Sephadex micro spheres (Hills & Babonneau, 1994; Hills, Wright, & Belton, 1989), porous silica (Hills, Belton, & Quantin, 1993; Hills, Wright, & Snaar, 1996), polymer hydrogels (Barbieri, Quaglia, Delfini, & Brosio, 1998; Brosio, Barbieri, & Gianferri, 2002), compartmentalized sub-cellular structures (Callaghan, 1991; Hills & LeFloc'h, 1994) and food systems (Gianferri, Maioli, Delfini, & Brosio, 2007; Gianferri, D'Aiuto, Curini, Delfini, & Brosio, 2007; Hills, 1998; Raffo, Gianferri, Barbieri, & Brosio, 2005). In addition, in native starch granules from corn, potato and pea, cross-polarization-magic-angle-spinning (CP MAS) methods and proton relaxation induced spectral editing (PRISE) techniques (Tang & Hills, 2003) are used to investigate the structural characteristics of the starch polysaccharides in different domains and dynamic states. Furthermore, it should be pointed out that to better interpret water protons relaxation data in greatly hydrated heterogeneous systems a complete and well-built model has been developed, based on the effect of diffusive and chemical exchange of water molecules (Hills, Takacs, & Belton, 1990; Hills et al., 1989). That model enables rationalizing relaxation data for water in heterogeneous systems and obtaining information on water dynamics and water microscopic distribution among various compartments.

In this paper NMR parameters known as solid to liquid ratio, proton transverse and longitudinal relaxation times of water saturated suspension of rice flour starch, measured at different temperatures, have been reported. The obtained data have been analysed and discussed on the basis of the diffusive and chemical exchange model in order to get a deeper knowledge of water distribution and dynamics inside starch granules, following swelling and gelatinisation processes.

2. Materials and methods

2.1. Materials

Natural white rice (Baldo, Japonica variety) was grown (2005 season) and processed from Rice Experimental Board (Ente Sperimentale Risi, Milano, Italy) in Italy. The rice was ground to a flour by sample mill (Cyclotec Tecator 1093, Foss S.p.A., Padova, Italy), then its composition was determined: moisture ($11\%_{w}$) was estimated according to the method described by Acquistucci, Bucci, Magrì, and Magrì (1991); protein ($7.1\%_{w}$), lipid ($0.66\%_{w}$) and ash ($0.4\%_{w}$) were determined using Italian Official methods of analysis (1994). The total starch ($87.4\%_{w}$) was determined according to the enzymatic method described by McCleary, Solah, and Gibson (1994). The amylose content (total $17.2\%_{w}$, free $15.6\%_{w}$) of starch was estimated by UNI ISO method (1991).

2.2. DSC measurements

DSC measurements were carried out by a Perkin Elmer DSC-7 Thermal Analysis System (Perkin Elmer Corporation, USA) equipped with multitasking software for instrument control and data analysis (in particular, by processing the calorimetric curve, it calculates the relative enthalpy variation connected with the different endo-exothermic events). Given amounts of rice flour (containing about 1.8 mg of starch) and the needed amount of distilled water to obtain a sample suspension with starch to water ratio of 1:3, were accurately weighted into an aluminium pan (Perkin Elmer DSC, pans 50 μl). The suspension was homogenized by stirring with a platinum needle and then, the pan was closed with an aluminium cover (Perkin Elmer DSC), sealed with a Perkin Elmer sealing press and left to equilibrate for 10 min at 30 °C. Scans were made at a temperature range 30–90 °C, in a nitrogen stream (flow rate 15 ml min^{-1}) and a heating rate of 10 °C min^{-1}. An empty pan (air) and indium were used as a reference and calibration standard, respectively. All measurements were performed in sixfold. To note that experimental data from DSC are affected by kinetics and thus may be limited to the experimental conditions used (e.g., moisture content, heating rate, time of starch–water contact before run). In particular, the 1:3 starch to water ratio was chosen in that representing the minimum quantity of water able to obtain a complete gelatinisation process. The heating rate of 10 °C min^{-1} was chosen because it assures a good reproducibility in peak quality, and the corresponding results can be compared to other data obtained in the literature at the same scanning rate (Kawaljit & Narpinder, 2007; Qi, Tester, Snape, & Ansell, 2003). The chosen 10 min time of starch–water contact before run is the minimum time allowing water to penetrate thoroughly inside the starch structure. It has to be pointed out that, even if water-rice flour suspensions are heterogeneous, the measured DSC profiles do not show any evidence of heterogeneity as reported in other systems (Tang, Covington, & Hancock, 2003). Likely, the difference of the thermal stability of the major components of starch does not reach the level to show resolved multiple peaks in DSC profiles. In any case, the measured homogeneous DSC profiles are in agreement with results reported in the literature for similar rice samples (Donovan, 1979; Iturriaga, Lopez, & Anon, 2004). The initial gelatinisation temperature ($T_{\text{initial}} = 55.1 \pm 0.1$ °C), the onset temperature ($T_{\text{onset}} = 58.7 \pm 0.1$ °C), the peak temperature ($T_{\text{peak}} = 65.1 \pm 0.3$ °C), and the gelatinisation temperature ($T_{\text{end}} = 76.7 \pm 0.1$ °C), as well as the gelatinisation enthalpy ($\Delta H_{\text{gelatinisation}} = 10.1 \pm 0.2\ \text{J g}^{-1}$) were measured by processing the obtained endothermic curve.

2.3. NMR measurements

All NMR measurements were performed on a Minispec mq 20 pulsed NMR spectrometer (Bruker Spectrospin Company, Milano, Italy), with an operating frequency of 20 MHz for protons (magnetic field strength: 0.47 T). The NMR spectrometer was equipped with an external thermostat (Julabo F25, Julabo Labortechnik GmbH, Seelbach, Germany) to maintain the desired temperature conditions. The measurements were taken at different temperatures ranging from 20 to 77 °C.

The starch–water mixture was prepared by mixing (stirring by hand with a glass spike) a known amount (accurately weighed) of rice flour (ca. 180 mg) with distilled water to reach the definite starch to water ratio (1:3, and 1:1.25 for T_2 dispersion curve experiments) in a NMR tube (outside diameter 10 mm). To prevent water evaporation, a teflon plug was inserted and the tube was then sealed by a laboratory film. Before NMR measurements, the tube was placed into the NMR probe as long as needed for thermal equilibration (ca. 30 or 60 min for measuring temperatures lower or higher than gelatinisation temperature, respectively).

The solid to liquid ratios were obtained by a single–pulse experiment (with a 90° pulse of 3.4 μs). The free induction decay (FID) signal was measured as an average of 64 repetitions and with a recycle delay (RD) of 10 s to assure complete relaxation of nuclear spins between repetitions (ca. 5–10 T_1). The FID amplitude immediately registered after a dead time of 4–5 μs can be considered proportional to the proton number in the measured volume, both in the liquid and solid phase, while the FID amplitude measured after 55 μs is proportional to the proton number in the liquid phase. The relative amount of the solid phase, measured in terms of proton content, is S/L, where S and L are the signals of the protons in the solid and liquid phase, respectively. S was determined from the initial rapid decay (until ca. 20 μs), and L from a decay at a time longer than ca. 55 μs, assuming both linearly correlated, by extrapolation to the pulse end. The relative content of the solid phase by weight (SW) in the sample was calculated as

$$\text{SW} = \frac{\frac{L}{S}}{\frac{L}{S} + k(1 - \frac{L}{S})} \tag{1}$$

where the coefficient k is the relative abundance of protons in the liquid and solid phases. A k theoretical estimate of 2.58 can be made taking into account the different hydrogen abundance of starch, 7/162, compared to that one of water, 2/18 (Brosio, Conti, Lintas, & Sykora, 1978)

$$k = \frac{2/18}{7/162} = 2.58. \tag{2}$$

The transverse relaxation curves were measured by the Carr–Purcell–Meiboom–Gill (CPMG) sequence (Meiboom & Gill, 1958) with pulse spacing (τ_{cp}) between two following pulses of 0.04 ms. The first 400 data points of the relaxation curve were acquired after each echo signal (to detect the shortest T_2 value), while the other 2500 or 1000 data points (depending on whether measurements were performed before or after the gelatinisation temperature) were acquired after each 8th echo (to detect the longer T_2 values) as an average of 32 repetitions, and with a RD of 10 s, such to assure complete relaxation of the nuclear spins between repetitions (ca. 5–10 T_1).

In the CPMG experiments for determination of the transverse relaxation dispersion curves, the proton probe was thermostated at 20 °C and τ_{cp} was varied between 0.04 and 500 ms; a suitable number of data points to obtain a sampling space of ca. 620 ms were acquired, after each echo signal, as an average of 32 repetitions, and with a RD of 10 s.

The transverse magnetization decay curves were fitted to exponential decays using the equation

$$A(\tau) = \sum_i A_{(i)} \cdot \exp\left(-\frac{\tau}{T_{2(i)}}\right) + L_0 \tag{3}$$

where $A(\tau)$ is the echo amplitude at time τ, $T_{2(i)}$ and $A_{(i)}$ are the spin–spin relaxation time and amplitude, respectively, of component i, and the constant L_0 represents the decay curve noise.

The longitudinal relaxation times, T_1, were determined by the inversion recovery (IR) sequence (Ernst, Bosedhausen, & Wokaun, 1991). The recycle delay (RD) was 10 s, the first and last pulses spacing (τ) between the 180° and 90° pulses were 0.1 ms and 20 s, respectively. The IR sequence was recorded for 100 variable delay times, and eight scans were acquired for each measurement.

The relaxation recovery curves were fitted using a multi-exponential model according to the equation

$$M(\tau) = \sum_i M_{\infty(i)} \left[1 - 2 \cdot \exp\left(-\frac{\tau}{T_{1(i)}}\right)\right] \tag{4}$$

where $M(\tau)$ is the signal amplitude (as NMR signal percentage) at the time τ, $T_{1(i)}$ and $M_{\infty(i)}$ are the spin-lattice relaxation time and signal percentages, respectively, of component i.

3. Results and discussion

Proton transverse relaxation curves were measured by CPMG pulse sequence, at different temperatures, for water saturated suspension of starch, obtained by addition of excess of distilled cold water to rice flour, so to reach the definite 1:3 starch to water ratio, the latter chosen on the basis of the DSC study. The measured experimental curves did not show a single-exponential behaviour and were analysed for the presence of different components, which is expected for water–starch saturated systems. As an example Fig. 1 shows proton transverse magnetization decay curves of starch–water suspensions measured at the temperatures of 20 °C and 77 °C. The CPMG curves have been analysed for four and three exponential components, respectively. The obtained results are summarized in Table 1, where the different components T_2 values and their relative percentages, for increasing measuring temperatures (20–77 °C), are reported.

Firstly, it should be pointed out that up to 54 °C, the transverse magnetization relaxation decay can be analysed by a quadri-exponential curve, while, from 58 °C to 77 °C, best fit for relaxation data gave a tri-exponential behaviour. This highlights a substantial change of the water–starch system with temperature, that was also observed by DSC scanning as an endothermic transition at the onset temperature of 59 °C (shown in Fig. 2) and interpreted as ascribable to the starch gelatinisation process.

Moreover, the solid to liquid ratios of the different water–starch saturated samples were measured by the free induction decay signal (FID) analysis. This measuring method, based on the different decay rate of FID for a solid phase, i.e., few tens of microseconds, compared to that one for liquids, i.e., several milliseconds, was extensively described in a previous paper (Brosio et al., 1978) and was used to quantify the extent of starch gelatinisation in wheat starch (Hendes da Silva, Ciacco, Barberis, Solano, & Rettori, 1996). The solid to liquid ratio values, measured in terms of hydrogen abundance, S/L, were transformed in the quantity of more general interest, the relative content of solid phase by weight, SW, using a theoretical conversion factor $k = 2.58$, as explained by Eq. (2) in Section 2. The SW solid percentages for starch–water saturated samples, in a 20–77 °C temperature range, are shown in Fig. 2. It can be easily seen that, between 50 and 70 °C, the solid content of samples drops to a very low value due to transition to gel state of water–starch system, as already highlighted by DSC measurements and NMR transverse relaxation data.

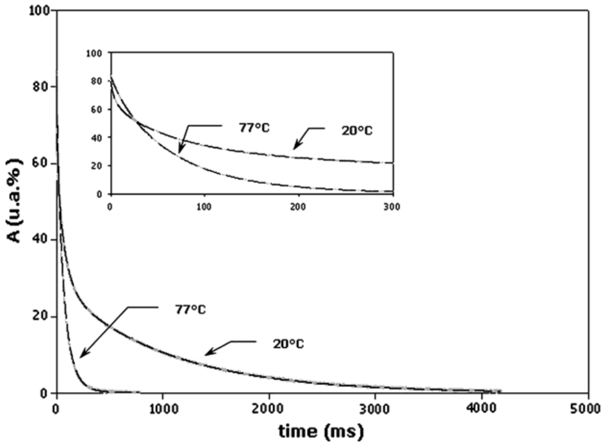

Fig. 1. Experimental CPMG decay curves of 1:3 starch–water saturated samples, at the temperature of 20 and 77 °C (grey points), and multiexponential decay best fits (black lines). Best fits of relaxation curves gives four components at 20 °C (coefficients of the regression: $P < 0.0001$, $R = 1.0000$ and $R^2 = 1.0000$), and three components at 77 °C (coefficients of the regression: $P < 0.0001$, $R = 1.0000$ and $R^2 = 1.0000$). P, R, and R^2 are probability density, regression equation coefficient, and correlation coefficient, respectively.

We shall go back to further discuss the effects of gelatinisation process on NMR measured relaxation curves. The assignment of the relaxation curve components, characterized by different intrinsic relaxation times, to several subpopulations of water within water–starch system is not so obvious, due to the micro structural complexity of starch. Actually, in heterogeneous systems relaxation is affected by the interior structure as a result of water-macromolecules interactions. Therefore, not only translation and rotation of water molecules affect relaxation, but also a diffusive and chemical exchange process between water molecules and biopolymers, or other solutes, strongly contributes to T_2 and T_1 values (Hills et al., 1990). Clearly, the most relevant effect is that of starch, being the more abundant biopolymer in the system; the contribution of protein (7.1‰$_w$) to exchange processes, if present, may be assumed in the first approximation as the same to that of starch, while, lipids (0.66‰$_w$) do not contribute at all to the exchange process. In Fig. 3 the different components T_2 values of the water–starch saturated samples, measured in the temperature range 20–80 °C, are reported. As shown for component a, T_2 values linearly increase with temperature according to a dominant dipolar mechanism contribution, while for components b and c, the T_2 values trend is characterized by a flat minimum, according to a diffusive exchange mechanism contribution. Finally, T_2 values of component d, almost independent of temperature, evidence a strong interaction between water molecules and starch, and this suggests that diffusion is not playing a significant role, supporting a fast chemical exchange assumption (Latour, Kleinberg, & Sezginer, 1991).

Hence, with reference to the data reported on the first row of Table 1, $T = 20$ °C, the slowly relaxing component, $T_{2,a} = 1056 \pm 2$ ms, is associated to extra granular bulk water molecules, whose motion is in no way affected by the presence of starch granules. These water molecules have essentially the same dynamic properties as water in a solution, i.e. characterized by an isotropic motion, as supported by T_1/T_2 ratio value near to unity ($T_1 = 1076 \pm 48$ ms, see Table 2). This assignment is furthermore confirmed by the fact that this component vanishes by lowering the water content of samples down to a

Table 1
Proton transverse relaxation time values (T_2) and signal percentages at different measuring temperatures (20–77 °C) in 1:3 starch–water samples

T (°C)	$T_{2,a}$ (ms)	% a	$T_{2,b}$ (ms)	% b	$T_{2,c}$ (ms)	% c	$T_{2,d}$ (ms)	% d
20	1056 ± 2	35 ± 1	85 ± 1	33 ± 1	32 ± 1	17 ± 1	3.1 ± 0.1	15 ± 1
31	1370 ± 5	40 ± 1	74 ± 1	33 ± 1	26 ± 1	13 ± 1	3.0 ± 0.1	14 ± 1
40	1623 ± 9	37 ± 1	70 ± 1	34 ± 1	25 ± 1	14 ± 1	2.8 ± 0.1	15 ± 1
54	689 ± 23	12 ± 1	73 ± 1	44 ± 1	22 ± 1	26 ± 1	2.5 ± 0.1	18 ± 1
58	–	–	72 ± 1	50 ± 1	24 ± 1	35 ± 1	3.0 ± 0.1	15 ± 1
60	–	–	67 ± 1	48 ± 1	25 ± 1	38 ± 1	3.3 ± 0.1	14 ± 1
64	–	–	69 ± 1	62 ± 1	29 ± 1	32 ± 1	3.2 ± 0.1	6 ± 1
77	–	–	82 ± 1	62 ± 1	37 ± 1	35 ± 1	2.7 ± 0.2	3 ± 1

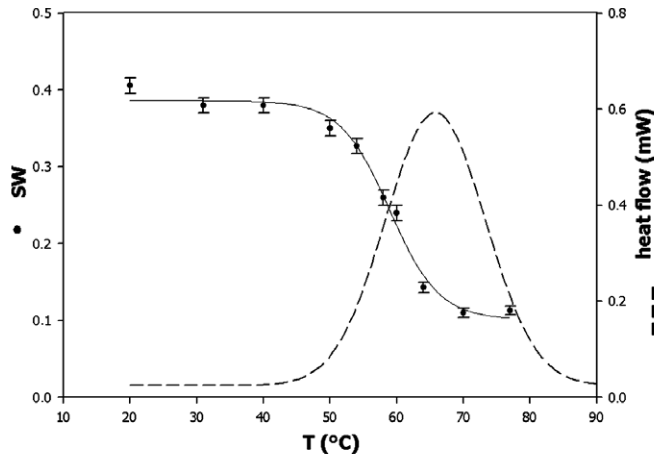

Fig. 2. The solid percentages SW (solid line), and DSC endothermic peak (dotted line) for 1:3 starch–water saturated samples, at the temperature range of 20–90 °C. Solid line is guide to eyes only.

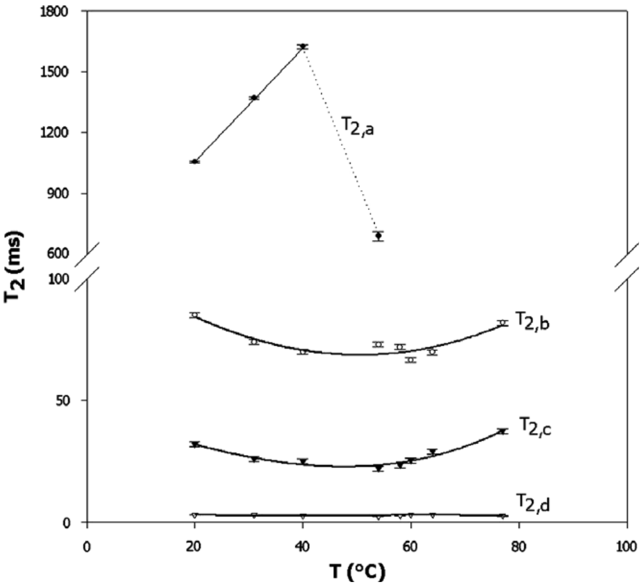

Fig. 3. The different components T_2 values of the starch–water saturated samples (1:3 starch to water ratio), at the temperature range of 20–80 °C. Lines are guides to eyes only.

Table 2
Proton longitudinal relaxation time values (T_1) and signal percentages at different measuring temperatures (20–77 °C) in 1:3 starch–water samples

T (°C)	$T_{1,a}$ (ms)	% a	$T_{1,b}$ (ms)	% b
20	1076 ± 48	53 ± 1	147 ± 9	47 ± 1
31	1465 ± 66	50 ± 1	189 ± 8	50 ± 1
40	1848 ± 75	46 ± 1	217 ± 7	54 ± 1
54	1657 ± 170	30 ± 1	290 ± 13	70 ± 1
58	–	–	417 ± 7	–
60	–	–	447 ± 7	–
64	–	–	512 ± 11	–
77	–	–	420 ± 9	–

water saturation capacity of starch – ca. 45% (data not shown).

As it concerns the other relaxation components, the measured T_2 values, low compared to those of bulk water, clearly reflect an interaction of water molecules with starch and are to be assigned to water inside the starch granule.

The fast relaxation component, $T_{2,d} = 3.1 \pm 0.1$ ms, can be associated to water molecules, trapped within amylopectin or amylose helices in the crystalline phase of starch. This transverse relaxation time is essentially dominated by a fast chemical exchange with amylopectin or amylose exchangeable OH protons, which have a T_2 of the order of magnitude of ten microseconds (Tang et al., 2001).

The intermediate relaxing components, $T_{2,b} = 85 \pm 1$ ms and $T_{2,c} = 32 \pm 1$ ms, can be ascribed to non-exchangeable hydrogens of more mobile sub-granular starch components – amylose and amylopectin CH protons (Tang et al., 2001) – as well as to water protons interacting by diffusion with structured amylose and amylopectin in the starch amorphous regions. These water molecules are likely to interact with the polysaccharide structure by diffusion from the bulk to the biopolymer interface acting as a relaxation sink, while T_2 values are controlled by diffusive exchange. The observation of two different T_2 values may be consistent both with the difference in the mobility and crystallinity of amylose and amylopectin chains or by a polydispersion in the radius (and geometry) of diffusive domains (Hills et al., 1998) following starch swelling.

Proton NMR relaxation data of saturated water–starch suspensions can be discussed on the basis of the diffusive and chemical exchange model, in order to explain the multiexponentiality of transverse relaxation decay curves, and also to rationalize the enhanced relaxation rate (lower T_2 values) measured in water molecules characterized by a different dynamic state in the different sub-granular starch compartments (Hills, Cano, & Belton, 1991).

As regards the relaxation rate of water interacting through a fast chemical exchange process with exchangeable OH protons of starch, the measured $T_{2,d}$ value is given by the equation (Hills et al., 1989)

$$\frac{1}{T_{2,d}} = \frac{1}{T_{2,\mathrm{w}}} + \frac{f \cdot P_{\mathrm{exch}}}{T_{2,\mathrm{exch}} + k_{\mathrm{exch}}^{-1}} \tag{5}$$

where $T_{2,\mathrm{w}}^{-1}$ is the water protons intrinsic relaxation rate that is likely to be given by the measured $T_{2,a}$ value of bulk water external to starch domains; P_{exch} and $T_{2,\mathrm{exch}}$ are starch exchangeable hydrogens molar fraction and intrinsic transverse relaxation time, respectively; k_{exch} is the pseudo-first order rate constant of chemical exchange between water and biopolymer; finally, f factor is the fraction of starch protons effectively accessible to exchange with water.

P_{exch} value can be calculated taking into account the sample composition and relative concentration of amylose and amylopectin starch fractions that have been measured by UNI ISO method (1991). According to the measured values and considering the number of exchangeable hydrogens per monomeric unit for amylopectin (8/485) and amylose (3/162), the $P_{\mathrm{exch}} = 0.0493 \pm 0.0004$ is derived.

The k_{exch} value can be obtained by exploiting the dependence of the transverse relaxation time on the inter-pulse spacing τ_{cp} value in a CPMG sequence (Hills et al., 1991; Hills, Takacs, & Belton, 1989). For nuclei exchanging between two sites, in fact, from mid-point of plot reporting relaxation rate as a function of inter-pulse CPMG spaces, the rate constant of the exchange process can be derived. Fig. 4 shows the obtained curve of $T_{2,d}^{-1}$ values vs. τ_{cp}^{-1} for the water–starch sample measured at $T = 20$ °C. The vertical dash line, located at the step mid-point, corresponds to $\tau_b^{-1} = k_{\mathrm{exch}}$ (Hills et al., 1989), wherefrom a k_{exch} value of $(11.546 \pm 0.1)10^3\ \mathrm{s}^{-1}$ is obtained.

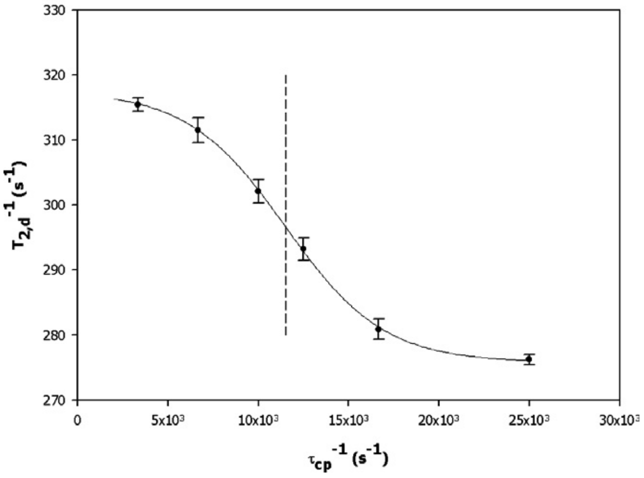

Fig. 4. $T_{2,d}^{-1}$ relaxation rates measured by CPMG sequence at different τ_{cp} values (from 0.04 to 400 ms) in for 1:1.25 starch–water samples, at 20 °C. The vertical dashed line corresponds to the step mid-point, $\tau_b^{-1} = k_{\mathrm{exch}}$ (Hills, 1991), from which a value $k_{\mathrm{exch}} = (11.5 \pm 0.1)10^3\ \mathrm{s}^{-1}$ was obtained. Solid line is a guide to eyes only.

Finally, we assumed for $T_{2,\mathrm{exch}}$ the value as measured by Tang et al., 2001). The authors found in the FID from the native potato starch dispersion in D_2O, a main relaxation time Gaussian component at ca. 20 μs, corresponding to hydrogens of rigid amylopectin and amylose in the semi-crystalline lamella (Tang et al., 2001). Based on this finding, an f value of 0.69 ± 0.02 has been derived. That means that, due to the helices structure of amylopectin and amylose crystalline phase, only about 69% exchangeable starch protons are actually accessible to water molecules. In fact, it is realistic to speculate that some of starch exchangeable hydrogens are not available to chemical exchange with water protons because directly used in the crystalline lattice formation.

As far as the measured entrapped water in the different sub-granular starch domains, whose relaxation rate is essentially controlled by a diffusive exchange from the bulk to biopolymer interface, if relaxation on the surface is fast compared with diffusion to surface – diffusion limited relaxation regime – the observed water transverse relaxation rate is independent of the effective surface relaxation strength and it is given by the equation (Belton, Hills, & Raimbaud, 1988)

$$\frac{1}{T_{2,\mathrm{oss}}} = \frac{1}{T_{2,\mathrm{w}}} + \frac{\pi^2 D}{4a^2} \tag{6}$$

where $T_{2,\mathrm{w}}^{-1}$ and D are water protons intrinsic relaxation rate and self-diffusion coefficient, respectively, a is the radius of water filled compartment bound by surfaces relaxation sink "$2a$" apart. On a smaller scale, a may describe the distance of the outside faces of super-junction zones in the interstitial spaces of gels (Belton et al., 1988). Assuming that water diffusion is in the order of that for bulk water, $D_{\mathrm{water}}^{20\,^{\circ}\mathrm{C}} = 2.023 \times 10^{-9}\ \mathrm{m}^2\ \mathrm{s}^{-1}$, Eq. (6) gives a maximum diffusive distance (Belton et al., 1988), a, of 21 μm and 13 μm for water molecules, whose $T_{2,b}$ and $T_{2,c}$ values are 85 ms and 32 ms, respectively. These values of the diffusive domain size are consistent with the results of Ohtsuka, Watanabe, and Suzuki (1994) who, by fitting echo attenuation data in diffusion measurements, according to the Meerwall Ferguson's model, found pores dimension in starch gels in the range 10–20 μm, and with dispersion of spatial heterogeneity, 5–20 μm, reported for a variety of starch-based systems through SEM measurements (Charles, Kao, & Huang, 2003) and diffusion data (Hills, 1998; Meerwall & Ferguson, 1981). This is consistent to the structure reported by Gallant, Bouchet, and Baldwin (1997) for native starch, in which polysaccharides assembled together to form granules with a diameter of 1–100 μm, which consists of crystalline hard shells and semi-crystalline soft shells in alternating fashion, characterized by different T_2 values (Tang & Hills, 2003).

So far the analysis of proton transverse magnetization decay curves has enabled having a more detailed insight into water dynamics and microscopic distribution of water–starch samples.

When a starch suspension is heated in a water excess, the gelatinisation process takes place, and up to the temperature of 40 °C, as shown by the T_2 relaxation data reported in Table 1, a substantial change is observed in the system. Only $T_{2,a}$ values, associated to extra granular water molecules get higher, linearly increasing with temperature, according to a dominant dipolar mechanism contribution to bulk water relaxation. At the temperature of 54 °C the percentage of extra granular water considerably decreases until disappearance of external water fraction, as observed at the temperature of 58 °C. At the same time, water populations associated to water molecules interacting with amorphous regions inside starch granules are observed to increase. The percentages of *b* and *c* components (ca. 33% and 15%, respectively, until 40 °C) increase by 62% and 35%, respectively, at the temperature of 77 °C. Finally, it is further to be evidenced that the population of water molecules associated to the starch crystalline region, whose $T_{2,d}$ value is determined by a chemical exchange process with double helices OH protons, decreases from 15% to 3% at a 60–77 °C temperature range.

An earlier hypothesis for the starch gelatinisation mechanism as reported in the literature (Donovan, 1979; Jenkins & Donald, 1998) presumed that the first stage of gelatinisation in excess of water is the uptake of water by the amorphous background region of the starch granules, followed by their subsequent rapid expansion. This swelling exerts a strong destabilizing effect on the crystallites contained within the crystalline lamellae. At the end, these crystallites are disrupted and the crystallinity is progressively lost. The evolution with temperature of the different water molecules starch populations, i.e., increase of the *b* and *c* components percentage and decrease of *d* components percentage, as deducted by our interpretation of the relaxation data, is in agreement with this description.

To complete our study, proton longitudinal magnetization recovery curves were measured by IR pulse sequence, for the same water–starch suspensions, at the temperature range of 20–77 °C. The analysis of longitudinal magnetization decay curves measured for the same water–starch samples gave two components before gelatinisation (temperature lower than 54 °C), and only one component for the gel state. The obtained results are summarized in Table 2, where the different components T_1 values and their relative percentages are reported. Because of the lower number of experimental points measured by IR sequence, with respect to those sampled by CPMG sequence, the exponentials curve deconvolution is less accurate, thus it was not possible to estimate the same number of T_2 components for longitudinal relaxation. Furthermore, being that T_1 in heterogeneous systems is higher than T_2, the exchange processes play a different role in determining the multiexponentiality of the recovery curve and resolution with respect to T_1, which is generally lower than that of T_2. For this reason, even if different T_2 values are generally measured for water in heterogeneous systems, T_1 curves are single-exponential (Hills et al., 1998).

The data reported in Table 2, however, provide evidence that in the swelling process we distinguish between an external water associated to $T_{1,a}$ values, typical of free water, whose dominant relaxation mechanism is the dipolar one, and an internal water whose $T_{1,b}$ is determined by interaction with starch components. After gelatinisation only one T_1 value, associated to water in gel state is measured.

Our results seem to give a more detailed description of water distribution in starch granules with respect to the one reported in the literature involving differing amounts of weakly and strongly bound water (Charles et al., 2003; Le Boltan, Rugraff, Martin, & Colonna, 1998). Indeed, our description is based on the assumption that the different components in the relaxation curve are to be related to granule structure and not to the so-called bound water. That in agreement with the interpretation of Tang et al. (2000), which in native starch granules from corn, potato and pea have been found three transverse relaxation time components, associated with polysaccharides in the crystalline lamellae, polysaccharides in the amorphous lamellae and amylose in the gaps between amylopectin clusters and with mobile starch protons. However, some differences have been found: an improved resolution of the transverse relaxation decay curve, probably due a better choice of the measuring parameters in CPMG sequence, i.e., a τ_{cp} interpulse spacing of 40 μs (instead of 150 μs), allowed the quadri-exponential deconvolution of rice starch–water samples transverse relaxation curve. Finally, we think the components characterized by T_2 of the order of magnitude of tens milliseconds have to be associated to water molecules inside starch granules, even if for water concentrations lower than starch saturation capacity, a T_2 value of 50 ms may be also due to interstitial water molecules diffusing between the outside faces of super-junction zones of starch–water gel.

4. Conclusion

The potential of NMR relaxometry to investigate starch–water interaction has been fully exploited. By the analysis of proton relaxation curves different water populations in starch granules have been observed thus giving the possibility of a deeper insight into water dynamics and distribution in starch. Relaxation data have been interpreted on the basis of a diffusive and chemical exchange model, so as to allow not only explanation of the multiexponentiality of relaxation curves, but also quantitative rationalization of the absolute values of relaxation times, thereby providing additional information on the kinetic constant of water–starch chemical exchange, the fraction of starch protons effectively accessible to exchange with water and the dimension of water diffusion domains. The analysis of transverse relaxation curves of starch–water samples measured at different temperatures allowed following the uptake of water following swelling and gelatinisation processes by measuring the vanishing of extra granular water and change of percentages in internal water populations.

To conclude, it is finally to be underlined that the described low-resolution NMR method to investigate starch–water interaction is very suitable for better understanding the response of starch-based foods to physical or chemical processing, since low-resolution NMR measurements are rapid and do not require any pre-treatment of the sample to be investigated.

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